Contents

Appendix Tables

Appendix Table S1. The enriched gene sets upregulated in resistant SKMEL28 and WM3248 cells compared with parental cells in GSEA analysis. (Statistically significant gene sets were underlined.)

RANK	Gene Sets	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
1	BCAT_BILD_ET_AL_DN	44	0.51	1.44	<0.001	0.308	0.233
2	RB_DN.V1_DN	120	0.64	1.43	0.043	0.172	0.247
3	CORDENONSI_YAP_CONSERVED_SIGNATURE	57	0.77	1.42	<0.001	0.174	0.271
4	TGFB_UP.V1_UP	180	0.54	1.36	0.129	0.352	0.505
5	KRAS.50_UP.V1_DN	46	0.55	1.36	0.132	0.314	0.543
6	KRAS.300_UP.V1_DN	140	0.51	1.34	0.073	0.370	0.603
7	ESC_J1_UP_EARLY.V1_DN	169	0.42	1.31	0.045	0.492	0.623
8	CAMP_UP.V1_DN	192	0.47	1.31	<0.001	0.458	0.623
9	STK33_SKM_UP	267	0.44	1.3	<0.001	0.473	0.639
10	RB_P130_DN.V1_DN	125	0.39	1.3	<0.001	0.476	0.639
11	GLI1_UP.V1_UP	25	0.46	1.3	0.11	0.437	0.639
12	KRAS.KIDNEY_UP.V1_DN	133	0.52	1.3	0.085	0.409	0.639
13	ATF2_UP.V1_DN	178	0.48	1.29	<0.001	0.416	0.639
14	CYCLIN_D1_UP.V1_DN	187	0.38	1.29	0	0.395	0.639
15	KRAS.600_UP.V1_DN	278	0.44	1.29	0.22	0.378	0.639
16	P53_DN.V1_UP	189	0.64	1.28	0.143	0.363	0.639
17	ESC_V6.5_UP_EARLY.V1_DN	164	0.56	1.28	0.322	0.399	0.674
18	PRC2_EZH2_UP.V1_UP	185	0.44	1.27	0.045	0.397	0.674
19	PDGF_ERK_DN.V1_DN	144	0.39	1.27	0.063	0.377	0.674
20	PRC2_EDD_UP.V1_DN	186	0.43	1.27	0.018	0.375	0.674

Appendix Table S2. List of top 20 significantly enriched gene sets downregulated upon YAP/TAZ knockdown identified by pre-ranked GSEA .

RANK	Gene Sets enriched in resistant SKMEL28	ES	NES	NOM p-val	FDR q-val	FWER p-val
1	CSR_LATE_UP.V1_UP	0.58	6.17	<0.001	<0.001	<0.001
2	VEGF_A_UP.V1_DN	0.5	5.57	<0.001	<0.001	<0.001
3	RPS14_DN.V1_DN	0.51	5.51	<0.001	<0.001	<0.001
4	E2F1_UP.V1_UP	0.49	5.44	<0.001	<0.001	<0.001
5	GCNP_SHH_UP_LATE.V1_UP	0.5	5.39	<0.001	<0.001	<0.001
6	RB_P107_DN.V1_UP	0.53	5.23	<0.001	<0.001	<0.001
7	CORDENONSI_YAP_CONSERVED_SIGNATURE	0.69	5.08	<0.001	<0.001	<0.001
8	PRC2_EZH2_UP.V1_UP	0.43	4.76	<0.001	<0.001	<0.001
9	ERB2_UP.V1_DN	0.41	4.52	<0.001	<0.001	<0.001
10	GCNP_SHH_UP_EARLY.V1_UP	0.42	4.48	<0.001	<0.001	<0.001
11	MYC_UP.V1_UP	0.37	4.11	<0.001	<0.001	<0.001
12	CSR_EARLY_UP.V1_UP	0.38	4	<0.001	<0.001	<0.001
13	BMI1_DN_MEL18_DN.V1_UP	0.38	3.97	<0.001	<0.001	<0.001
14	HOXA9_DN.V1_DN	0.33	3.68	<0.001	<0.001	<0.001
15	TBK1.DF_DN	0.29	3.6	<0.001	<0.001	<0.001
16	RB_P130_DN.V1_UP	0.37	3.58	<0.001	<0.001	<0.001
17	EGFR_UP.V1_UP	0.29	3.36	<0.001	<0.001	<0.001
18	RB_DN.V1_UP	0.34	3.32	<0.001	<0.001	<0.001
19	SIRNA_EIF4GI_DN	0.37	3.32	<0.001	<0.001	<0.001
20	SRC_UP.V1_DN	0.31	3.31	<0.001	<0.001	<0.001

RANK	Gene sets enriched in resistant WM3248	ES	NES	NOM p-val	FDR q-val	FWER p-val
1	CSR_LATE_UP.V1_UP	0.68	7.23	<0.001	<0.001	<0.001
2	VEGF_A_UP.V1_DN	0.56	6.17	<0.001	<0.001	<0.001
3	RPS14_DN.V1_DN	0.54	5.89	<0.001	<0.001	<0.001
4	CORDENONSI_YAP_CONSERVED_SIGNATURE	0.76	5.52	<0.001	<0.001	<0.001
5	E2F1_UP.V1_UP	0.48	5.07	<0.001	<0.001	<0.001
6	GCNP_SHH_UP_LATE.V1_UP	0.48	5.04	<0.001	<0.001	<0.001
7	RB_P107_DN.V1_UP	0.51	5	<0.001	<0.001	<0.001
8	CSR_EARLY_UP.V1_UP	0.47	4.93	<0.001	<0.001	<0.001
9	PRC2_EZH2_UP.V1_UP	0.45	4.8	<0.001	<0.001	<0.001
10	BMI1_DN_MEL18_DN.V1_UP	0.46	4.64	<0.001	<0.001	<0.001
11	MYC_UP.V1_UP	0.4	4.26	<0.001	<0.001	<0.001
12	EGFR_UP.V1_UP	0.38	4.17	<0.001	<0.001	<0.001
13	BMI1_DN.V1_UP	0.41	4.13	<0.001	<0.001	<0.001
14	TBK1.DF_DN	0.33	4.03	<0.001	<0.001	<0.001
15	ERB2_UP.V1_DN	0.37	3.98	<0.001	<0.001	<0.001
16	HOXA9_DN.V1_DN	0.37	3.96	<0.001	<0.001	<0.001
17	KRAS.LUNG.BREAST_UP.V1_UP	0.39	3.94	<0.001	<0.001	<0.001
18	GCNP_SHH_UP_EARLY.V1_UP	0.37	3.87	<0.001	<0.001	<0.001
19	MTOR_UP.V1_UP	0.37	3.8	<0.001	<0.001	<0.001
20	P53_DN.V1_UP	0.34	3.8	<0.001	<0.001	<0.001

Appendix Table S3. List of siRNA sequences used in this study.

siRNA name	Sense strand sequence	Knockdown confirmation	Reference
YAP si#1	5'-GACAUCUUCUGGUCAGAGA dTdT-3'	WB	(Dupont et al, 2011)
TAZ si#1	5'-ACGUUGACUUAGGAACUUU dTdT-3'	WB	(Dupont et al, 2011)
YAP si#2	5'-CUGGUCAGAGAUACUUCUU dTdT-3'	WB	(Dupont et al, 2011)
TAZ si#2	5'-AGGUACUUCCUCAAUCACA dTdT-3'	WB	(Dupont et al, 2011)
c-MYC si#1	5'-AACGUUAGCUUCACCAACA dTdT-3'	WB	Dharmacon
c-MYC si#2	5'-CGAUGUUGUUUCUGUGGAA dTdT-3'	WB	Dharmacon
TESK1 si#1	5'-GACCCGUCCUCAAUAACAA dTdT-3'	qRT-PCR	Dharmacon
TESK1 si#2	5'-UGAACAAGCUCCCCAGUAA dTdT-3'	qRT-PCR	Dharmacon

siYT#1 indicates YAP si#1 plus TAZ si#1, and siYT#2 indicates YAP si#2 plus TAZ si#2.

Appendix Table S4. List of primary antibodies used in this study.

Antibody Name	Application	Dilution	Source	Manufacturer	Catalog Number
AKT	WB	1:1000	Rabbit	Cell Signaling Technology	#9272
phospho-AKT (Ser 473)	WB	1:1000	Rabbit	Cell Signaling Technology	#9271
BRAF	WB	1:500	Mouse	Santa Cruz Biotechnology	sc-5284
BrdU	IF	1:1,000	Rat	Abcam	ab6326
Cofilin	WB	1:1,000	Rabbit	Cell Signaling Technology	#5175
Phospho-Cofilin	WB	1:1,000	Rabbit	Cell Signaling Technology	#3313
c-Myc	WB	1:1,000	Rabbit	Abcam	ab32072
	IF	1:1,000	Rabbit	Abcam	ab32072
EGFR	WB	1:1,000	Rabbit	Cell Signaling Technology	#4267
FLAG	WB	1:1,000	Rabbit	Sigma-Aldrich	F7425
LaminB	WB	1:1,000	Rabbit	Abcam	Ab16048
ERK1/2	WB	1:1,000	Rabbit	Cell Signaling Technology	#9102
Phospho-ERK (Thr202/Tyr204)	WB	1:1,000	Rabbit	Cell Signaling Technology	#4377
Ezrin	IF	1:500	Rabbit	Cell Signaling Technology	#3145
YAP/TAZ	IF	1:500	Mouse	Santa Cruz Biotechnology	sc-101199
	WB	1:1,000	Mouse	Santa Cruz Biotechnology	sc-101199
Phospho-YAP	WB	1:1,000	Rabbit	Cell Signaling Technology	#4911
GAPDH	WB	1:1,000	Mouse	Santa Cruz Biotechnology	sc-59540

Appendix Table S5. List of primer sequences used for qRT-PCR.

Gene	Primer name	Primer sequence	Reference
GAPDH	GAPDH F	CAACGGATTTGGTCGTATTG	
	GAPDH R	GCAACAATATCCACTTTACCAGAGTTAA	
ANKRD1	ANKRD1 F	AGTAGAGGAACTGGTCACTGG	(Dupont et al, 2011)
	ANKRD1 R	TGGGCTAGAAGTGTCTTCAGAT	
c-MYC	c-MYC F	CTTCTCTCCGTCCTCGGATTCT	(Li et al, 2013)
	c-MYC R	GAAGGTGATCCAGACTCTGACCTT	
CTGF	CTGF F	AGGAGTGGGTGTGTGACGA	(Dupont et al, 2011)
	CTGF R	CCAGGCAGTTGGCTCTAATC	
CYR61	CYR61 F	CAGGACTGTGAAGATGCGGT	(Feng et al, 2014)
	CYR61 R	GCCTGTAGAAGGGAAACGCT	
SOX10	SOX10 F	CTTTCTTGTGCTGCATACGG	(Sun et al, 2014)
	SOX10 R	AGCTCAGCAAGACGCTGG	
MITF	MITF F	CCGTCTCTCACTGGATTGGT	(Sun et al, 2014)
	MITF R	TGGGTCTGCACCTGATAGTG	
TESK1	TESK1 F	AGGTCTACAAGGTTCGGCAC	(Kim et al, 2015)
	TESK1 R	GTGCACACAGACTCCCATGA	

Appendix Supplementary Methods

Reagents, cell culture, and PLX4032-resistance establishment

PLX4032 (Vemurafenib), MK-2206, and Erlotinib were purchased from Selleckchem. Cytochalasin D (Sigma-Aldrich) was treated either at 200 nM or 5 μM, and blebbistiatin (Enzo Life Science) was treated at 50 μM. SKMEL28 cells were acquired from ATCC, and WM3248 cells were acquired from Coriell Institute. SKMEL28 cells were grown in MEM (Welgene) supplemented with 10 % FBS (Welgene), and WM3248 cells were grown in 4:1 combination of MCDB-153 and L-15 (Welgene) supplemented with 2 % FBS, 1.68 mM $CaCl_2$, and 5 μg/ml insulin (Welgene). HEK293T cells were grown in DMEM (Welgene) supplemented with 10 % FBS. To establish PLX4032-resistant cell lines, SKMEL28 and WM3248 cells were continuously exposed to 2 μM of PLX4032 for 2 months. PLX4032 containing media were replaced every 3-4 days. Survived resistant cells were cultured in the presence of 2 μM PLX4032 for all maintenance procedure. For experiments, cells were seeded on dish (or chamber) without PLX4032, and PLX4032 or DMSO control was applied as described in figure legends.

Plasmid, transfection, and retroviral infection

8xGTIIC-luciferase cDNA was provided by Dr. Stefano Piccolo (University of Padua; Addgene plasmid # 34615), and MSCV-c-MYC-IRES-GFP was provided by Dr. John Cleveland (Moffitt Cancer Center; Addgene plasmid # 18119). Flag-YAP wild-type and flag-YAP-5SA cDNAs cloned into retroviral pMSCV-puro vector were provided by Dr. Dae-Sik Lim (KAIST). We generated siRNA-resistant pMSCV-puro flag-YAP-5SA vector by performing site directed mutagenesis that incorporates silent mutations on YAP siRNA target site (nucleotide 553-561: GGTCAGAGA> GGGCAGCGC). Mutagenesis was performed using QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent Technologies) according to the manufacturer's protocol, and substitution of nucleotides was confirmed by Sanger sequencing. Transfection of siRNAs was performed with Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's transfection protocol. The sequence of siRNAs used in this study is described in Appendix Table S3. Plasmids were transfected with Lipofectamine LTX and PLUS reagent (Invitrogen) according to the manufacturer's protocol, and cells were fixed 24 hr after transfection. For retroviral particle assembly, retroviral constructs (pMSCV-puro, MSCV c-MYC IRES GFP, pMSCV-puro flag-YAP, pMSCV-puro flag-YAP-5SA, or siRNA-resistant pMSCV-puro flag-YAP-5SA) and two packaging plasmids (pCMV-VSV-G and pCMV-Gag-Pol) were co-transfected to HEK293T cells with Lipofectamine LTX and PLUS reagent, and retroviral supernatant was collected 24 hr after transfection. The supernatants were filtered through a 0.45-μm filter, and infected to melanoma cells with 4 μg/ml Polybrene. For stable cell line establishment, cells were grown in culture media supplemented with 3 μg/ml of puromycin for selection.

Sequencing

For sequencing of BRAF V600E mutation (c.1799T>A) in SKMEL28 and WM3248 cells, we designed a primer set as follows: forward, 5'-TGCTTGCTCTGATAGGAAAATGAG-3'; reverse, 5'-GCAGCATCTC AGGGCCAAAA-3'. Genomic DNAs were extracted from parental and resistant SKMEL28 and WM3248 cells using GenEx^{TM} Cell Kit (GeneAll) according to the manufacturer's protocol. Sanger sequencing

procedures using the forward primer on genomic DNAs were performed by SolGent.

Immunofluorescence

For indirect immunofluorescence, cells were plated either in 8-well Lab-Tek II chamber slides (Nunc) or 96-well clear bottom plates (BD Falcon), and fixed with 4 % paraformaldehyde for 8 min at room temperature. After fixation, 0.1 % Triton X-100 (Sigma-Aldrich) was applied for permeabilization. Cells were incubated with primary antibodies for 1 hr at room temperature. Bound primary antibodies were detected by incubating with Alexa Fluor 488- or 594-conjugated secondary antibodies (Life Technologies) for 1 hr at room temperature. For staining actin filaments, Alexa Fluor 594-conjugated Phalloidin (Life Technologies) was used according to manufacturer's protocol. Primary antibodies and dilutions used in this study are described in Appendix Table S4.

Microscopy and image analysis

Fluorescence images were acquired using a DeltaVision Spectris Imaging System (Applied Precision) equipped with an Olympus IX70 inverted microscope, objective lens [10x (NA 0.4; UPlanSApo), 20x (NA 0.75; UPlanSApo), 40x (NA 1.35; UApo N340) and 60x (NA 1.42; PlanApoN)], CoolSNAP HQ2 CCD camera, and SoftWorx software (Applied Precision). Confocal images were acquired using a Zeiss LSM 780 confocal laser scanning microscope equipped with a Zeiss Axio Observer Z1 inverted microscope, objective lens [40x (NA 1.1; LD C-Apochromat) and 63x (1.4 NA, Plan-Apochromat)], 32-channel GaAsP spectral detector (Quasar), and ZEN software (Carl Zeiss). Vertical images of Figure 2E were generated by stacking deconvoluted images of melanoma cells with 0.3 μm z-step size, and processing by SoftWorx volume rendering tool. For cell spreading area measurement, SKMEL28 and WM3248 cells were seeded on 8-well Lab-Tek II chamber slides with 2500 cells/well density and fixed after 24 hr incubation. Cells were fixed with 4 % paraformaldehyde and stained with fluorescent wheat germ agglutinin conjugates (Molecular Probes). Immunofluorescence images were acquired, and cell spreading area was measured by ImageJ software. All images were processed and placed in figures using Adobe Photoshop CS6 (Adobe Systems).

Micropattern experiment

All micropatterns used in this study were produced by CYTOO (http://www.cytoo.com/). Cells were seeded on coverslip chips containing fibronectin-coated disc-shaped 1600 μm^2 micropatterns (CYTOOchips™ DC-L-FN) or on unpatterned Lab-Tek chamber slides. Cells were incubated for 30 min, and unattached cells were flushed by changing medium. After flushing, cells were further incubated for 3 hr for allowing spreading, and fixed with 4 % paraformaldehyde.

BrdU incorporation assay

Cell were seeded at 5000 cells/well in 8-well Lab-Tek II chamber slides and incubated for 24 hr with FBS-supplemented media. Cells were treated with DMSO or PLX4032 (2 μM) for 24 hr, and 3 μg/ml of BrdU (Sigma-Aldrich) was applied for 45 min before fixation. Cells were fixed with 4 % paraformaldehyde for 8 min at room temperature followed by 10 min permeabilization with 0.4 % Triton X-100 (Sigma-Aldrich).

Slides were treated with 2 N HCl for 30 min to denature DNA. After denaturation, cells were stained with anti-BrdU antibody.

Cell viability assay

Cells were seeded at 2500 cells/well in 96-well microplates (SPL). For PLX4032 dose-response analyses, cells were incubated for 24 hr after plating, and then treated with variable doses of PLX4032 for 72hr. Viable cells were quantified by 450 nm absorbance measurement 2hr after treatment of Cell Counting Kit-8 reagent (CCK8; Dojindo) following the manufacturer's recommendations. For PLX4032 dose-response analyses after siRNA transfection, cells were seeded with siRNAs (reverse transfection). After transfection for 72hr, cells were treated with PLX4032 for 72hr before CCK8 assay. For dose response curve analysis, sigmoidal dose-response curves were fitted to data and IC50 values of cells with control siRNA and YAP/TAZ siRNA knockdown were compared using extra sum-of-squares F test by GraphPad Prism version 6 for Windows (GraphPad Software). P-value <0.05 indicates that IC50 is different between dose-response curves. For cell viability time-course analyses after YAP/TAZ knockdown, cells were transfected with siRNA for 48hr, and then treated with PLX4032 (2 μM). Cell viability was measured at 24, 72, 96, and 120 hr after drug treatment. Proportion of cell viability at each time point compared to initial cell viability were compared between melanoma cells with control siRNA and YAP/TAZ siRNA knockdown by Student's t-test.

Immunoblotting

For immunoblotting, cells were lysed with RIPA lysis buffer [20 mM HEPES (pH 7.5), 150 mM NaCl, 1 % NP-40, 0.25 % sodium deoxycholate, and 10 % glycerol] supplemented with protease and phosphatase inhibitors (Merck Millipore) on ice. Cell lysates were sonicated with a Branson Digital Sonifier 250 (Branson Ultrasonics) and centrifuged (13,000 rpm) for 10 min at 4 °C,. Protein concentrations of the supernatants were quantified using detergent-insensitive Pierce BCA protein assay kit (Thermo Scientific). Lamni buffer [62.5 mM Tris, 2 % SDS, 5 % 2-mercaptoethanol, 10 % glycerol, and 0.02 % bromophenol blue] was added, and lysates were denatured at 95 °C for 5 min. Aliquots of each protein lysate (10–20 μg) were applied to SDS–polyacrylamide gel electrophoresis for 90 min at 110 V. After electrophoresis, proteins were transferred to nitrocellulose membranes for 80 min at 300 mA, and incubated for 30 min with blocking solution (TBST buffer with 5 % skim milk). For phosphorylated protein detection, membranes were blocked with 2 % BSA in PBS. Blocked membranes were incubated with primary antibodies overnight at 4 °C, and then incubated with peroxidase-coupled secondary antibody (Santa Cruz Biotechnology) for 1 hr at 4 °C. Target proteins were detected using enhanced chemiluminescence western blot detection solution (Thermo Scientific).

Subcellular fractionation

Nuclear and non-nuclear fractional lysates were prepared using NE-PER nuclear and cytoplasmic extraction reagent (Thermo Scientific) according to the manufacturer's instructions. Cells were harvested for lysis by cell scrapers, instead of trypsin-EDTA, because changes in cell morphologies and mechanical tension during trypsinization process can affect the localization of YAP/TAZ.

Quantitative RT-PCR

Total RNA was extracted from cells using RNeasy kit (Qiagen) according to the manufacturer's instructions. A total of 1μg of extracted RNA was annealed with oligo dT primer (Roche Life Science), and reverse transcribed to cDNA using M-MLV reverse transcriptase (Promega) in the presence of RNasin Plus RNase Inhibitor (Promega). cDNA was mixed with primers and iQ SYBR Green Supermix (Bio-Rad), and mRNA expression levels were measured by real-time qRT-PCR on CFX96 system (Bio-Rad). The primers were designed using Primer-BLAST (Ye et al, 2012) or adopted from previously published studies. The list of qRT-PCR primers used in this study is described in Appendix Table S5. Primer reaction specificity was confirmed by both agarose gel electrophoresis and melting curve analysis. Relative gene expression was analyzed by ΔΔC(t) method using the CFX Manager software (Bio-Rad).

Luciferase assays

SKMEL28 and WM3248 cells were plated on 96-well plates at 5000 cells/well, and co-transfected with 8XGTIIC-luciferase vector (1 μg/ml) and pcDNA3.1-His-lacZ (1 μg/ml) for 24hr using Lipofectamine LTX and PLUS reagent. Cells were harvested with reporter lysis buffer, and luciferase activity was measured by luciferase assay kit (Promega) and TriStar LB941 Multimode Microplate Reader (Berthold) according to the manufacturer's instruction. β-Galactosidase activity was also measured using a β-Galactosidase enzyme assay system (Promega) for transfection efficiency normalization.

Expression microarray experiment

Total RNAs were extracted from melanoma cells using the RNeasy kit (Qiagen). RNA purity and integrity were evaluated by ND-1000 Spectrophotometer (NanoDrop) and Agilent 2100 Bioanalyzer (Agilent Technologies). Total RNA was amplified and purified using TargetAmp-Nano Labeling Kit for Illumina Expression BeadChip (EPICENTRE) to yield biotinylated cRNA according to the manufacturer's instructions. Briefly, 200 ng of total RNA was reverse-transcribed to cDNA using a T7 oligo(dT) primer. Second-strand cDNA was synthesized, in vitro transcribed, and labeled with biotin-NTP. After purification, the cRNA was quantified using ND-1000 Spectrophotometer. 750 ng of labeled cRNA samples were hybridized to Human HT-12 v4.0 Expression Beadchip for 17 hr at 58°C, according to the manufacturer's instructions (Illumina). Detection of array signal was carried out using Amersham fluorolink streptavidin-Cy3 (GE Healthcare Bio-Sciences) following the bead array manual. Arrays were scanned with an Illumina bead array Reader confocal scanner according to the manufacturer's instructions.

Expression microarray data analysis

The quality of hybridization and overall chip performance were monitored by visual inspection of both internal quality control checks and the raw scanned data. Raw data were extracted using the software provided by the manufacturer (Illumina GenomeStudio v2011.1; Gene Expression Module v1.9.0). Array probes were transformed by logarithm and normalized by quantile method. Statistical significance of the expression data was determined using local-pooled-error (LPE) test and fold change. False discovery rate (FDR) was controlled by adjusting p-value using Benjamini-Hochberg algorithm. The cutoff for significantly downregulated or upregulated hits was defined as fold change >2.0 and p-value <0.05.

DAVID gene enrichment and functional annotation analysis was performed for characterizing (i) significantly altered genes both in resistant SKMEL28 and WM3248 cells compared to each parental cells, and (ii) significantly downregulated genes upon YAP/TAZ siRNA knockdown, using Gene Ontology database (GOTERM_BP_FAT, GOTERM_MF_FAT, and GOTERM_CC_FAT). The list of significantly downregulated genes upon YAP/TAZ siRNA knockdown was submitted to TransFind algorithm (Kiełbasa et al., 2010) to query enriched transcription factor motifs. Promoter regions of 800 upstream to 200 downstream of transcription start sites (1000 nucleotides) were analyzed using TRANSFAC highest information database version 2009.04. To test whether YAP signature is enriched on resistant cells compared with parental cells, GSEA (Subramanian et al, 2005) using C6 MSigDB gene set database (C6 : collection of oncogenic signature) was performed, comparing parental and resistant cells of SKMEL28 and WM3248. To identify enriched oncogenic signature gene sets suppressed by YAP/TAZ knockdown, pre-ranked GSEA was performed for SKMEL28 and WM3248, respectively, using C6 MSigDB gene set database in the ranked gene list in the order of downregulation fold change upon YAP/TAZ knockdown.

Kinome siRNA library screening experiment

A kinome-wide siRNA library targeting 607 human kinases was purchased from Dharmacon in 384-well plate format. Four different siRNAs targeting each kinase were pooled. Polystyrene flat bottom 384-well plates (Greiner) were spotted with 3 ul of 0.25 μM siRNA library pools (62.5 nM for each siRNA) using the Biomek FX Laboratory Automation Workstation (Beckman Coulter) with 384 multichannel pod. Hexaplicates of control siRNA and triplicates of YAP/TAZ siRNA were also included in the plates as a negative and a positive control, respectively. 0.1 μl of Lipofectamine RNAiMAX dissolved in 7 μl of Opti-MEM (Gibco) was mixed in assay plates to perform reverse transfection with final total siRNA concentration of 15 nM. Resistant WM3248 cells were suspended in MCDB153/L15/$CaCl_2$/Insulin media supplemented with 2 % FBS, and seeded onto assay plates using MultiFlo Microplate Dispenser (Bio-Tek) at 1000 cells/well with final volume of 50 ul. PLX4032 was added 48 hr after transfection, and cells were further incubated for 72 hr. To measure cell viability, the half of medium was aspirated by ELx405™ Select Deep Well Microplate Washer (Bio-Tek), and CCK8 reagent was applied for 2 hr. Cell viability was measured by 450 nm absorbance on Mithras multimode microplate reader (Berthold).

Kinome siRNA library screening data analysis

Cell viability data of target siRNA from two biologic replicates were normalized by division of each target siRNA viability score by the mean control siRNA viability (hexaplicate) of each replicate. Z-scores of normalized target siRNA viability were calculated for each replicates. siRNA targets with Z scores < -2 in both replicates were considered as significant synthetic lethal hits, and siRNA targets with Z scores greater than 2 were considered as growth promoting hits.

TESK1 genetic alteration in human melanoma samples

The frequency of TESK1 genetic alterations in published human melanoma sample database was evaluated using cBioPortal for Cancer Genomics (Cerami et al, 2012; Gao et al, 2013). TESK1 alterations including mutations, amplification, and mRNA upregulations were searched in 278 complete tumor sample data from

skin cutaneous melanoma database of The Cancer Genome Atlas Network (TCGA).

Quantification and statistical analysis

The quantification of YAP/TAZ localization was performed by inspecting at least 150-200 cells stained with anti-YAP/TAZ immunofluorescence, using ImageJ software. YAP/TAZ localization was classified into three categories: nuclear (higher nuclear intensity than cytoplasm), nucleocytoplasmic (equal intensity of nucleus and cytoplasm), and cytoplasmic (higher cytoplasmic intensity than nucleus). The data of qRT–PCR and luciferase assay were normalized by dividing all values of control and treatment groups by the mean of the control. Data analysis was performed using GraphPad Prism version 6 for Windows (GraphPad Software), and statistical significance was considered when p-value was <0.05 in an unpaired Student's t-test. Paired t-test was used for evaluating relative survival after drug treatment or siRNA transfection (survival values were normalized by control cell survival value before the test).

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